SAMPLE
Matrix: solutions
Sample preparation: Inject a 40 μL aliquot of an aqueous solution.

HPLC VARIABLES
Column: 250 × 4.6 5 μm LiChrospher C18
Mobile phase: MeCN:KH_2PO_4 20:80, pH 5.5
Column temperature: 37.5
Flow rate: 1.7
Injection volume: 40
Detector: UV 220

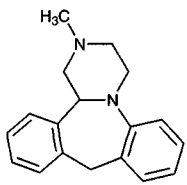
CHROMATOGRAM
Retention time: 7.7
Internal standard: mezlocillin

OTHER SUBSTANCES
Simultaneous: piperacillin

KEY WORDS
mezlocillin is IS

REFERENCE
Kinzig,M.; Sörgel,F.; Brismar,B.; Nord,C.E. Pharmacokinetics and tissue penetration of tazobactam and piperacillin in patients undergoing colorectal surgery, *Antimicrob.Agents Chemother.*, **1992**, *36*, 1997–2004.

Mianserin



Molecular formula: $C_{18}H_{20}N_2$
Molecular weight: 264.37
CAS Registry No.: 24219-97-4, 21535-47-7 (HCl)
Merck Index: 6260
Lednicer No.: 2 451

SAMPLE
Matrix: Blood, tissue, urine
Sample preparation: Serum, urine. 500 μL Serum or urine + 100 μL 2 μg/mL diazepam + 200 μL 20% sodium carbonate + 500 μL water + 3 mL n-hexane:isoamyl alcohol 98.5:1.5, mix for 2 min, centrifuge at 1200 g for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, inject a 10 μL aliquot. Tissue. Homogenize 1 g sample with 9 mL 100 mM HCl and 100 μL 20 μg/mL diazepam, centrifuge at 15000 g for 10 min. Add 500 μL 20% sodium carbonate and 4 mL n-hexane:isoamyl alcohol 98.5:1.5 to 1 mL of the supernatant, mix for 5 min. Remove the organic phase and evaporate it under a gentle stream of nitrogen at about 40°. Dissolve the residue in 100 μL mobile phase, filter by microconcentrator (Microcon-30, Grace). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 2 μm TSK gel Super-Octyl (A) or 100 × 4.6 5 μm Hypersil MOS-C8 (B), (Yokogawa, Japan)
Mobile phase: MeOH:20 mM pH 7 KH_2PO_4 60:40
Flow rate: 0.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 9.3 (A), 13.5 (B)
Internal standard: diazepam (4.4, A)

Limit of quantitation: 50 ng/mL (serum, urine), 500 ng/mL (tissue)

OTHER SUBSTANCES

Extracted: amitriptyline, amoxapine, clomipramine, desipramine, dothiepin, doxepin, imipramine, maprotiline, melitracen, nortriptyline

Noninterfering: barbital, carbamazepine, ethosuximide, hexobarbital, lofepramine, pentobarbital, phenobarbital, phenytoin, primidone, sulpiride, trimethadione, trimipramine

KEY WORDS

serum; brain; liver

REFERENCE

Tanaka,E.; Terada,M.; Nakamura,T.; Misawa,S.; Wakasugi,C. Forensic analysis of eleven cyclic antidepressants in human biological samples using a new reversed-phase chromatographic column of 2 μm porous microspherical silica gel, *J.Chromatogr.B*, **1997**, *692*, 405–412.

SAMPLE

Matrix: blood

Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18

Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)

Column temperature: 30

Flow rate: 0.8

Injection volume: 50

Detector: UV 279

CHROMATOGRAM

Retention time: 6.02

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; aceprometazine; glibenclam-

ide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.787

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: bulk

Sample preparation: Dissolve 2 mg mianserin in 1 mL EtOH, dilute with 4 mL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Chiralcel OD or 250 × 4.6 Chiralpak AD

Mobile phase: EtOH:n-hexane 95:5 or n-hexane:2-propanol 90:10

Flow rate: 0.5 or 1

Injection volume: 20

Detector: UV 250

KEY WORDS
chiral

REFERENCE
Selditz,U.; Liao,Y.; Franke,J.P.; de Zeeuw,R.A.; Wikström,H. Direct enantiomeric separation of mianserin and 6-azamianserin derivatives using chiral stationary phases, *J.Chromatogr.A*, **1998**, *803*, 169–177.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

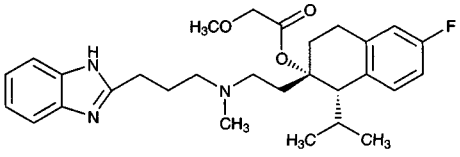
HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE
Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Mibefradil



Molecular formula: $C_{29}H_{38}FN_3O_3$
Molecular weight: 495.64
CAS Registry No.: 116644-53-2, 116666-63-8 (2.HCl)
Merck Index: 6261

SAMPLE
Matrix: blood
Sample preparation: Add 100-30 ng/mL IS, extract either using dichloromethane or using a Bond-Elut C2 SPE cartridge.

HPLC VARIABLES
Column: 3μm C6 (Gromsil or Spherisorb)
Mobile phase: MeCN:9.5 mM pH 3.9 phosphate buffer 55:45 to 70:30
Flow rate: 1
Detector: F ex 270 em 300

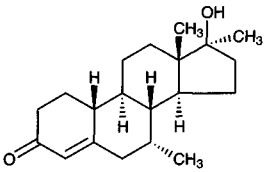
CHROMATOGRAM
Internal standard: Ro 40-6792

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; marmoset; cynomolgus monkey; human; plasma; pharmacokinetics; SPE

REFERENCE
Wiltshire,H.R.; Sutton,B.M.; Heeps,G.; Betty,A.M.; Angus,D.W.; Harris,S.R.; Worth,E.; Welker,H.A. Metabolism of the calcium antagonist, mibefradil (POSICOR, Ro 40-5967). Part III. Comparative pharmacokinetics of mibefradil and its major metabolites in rat, marmoset, cynomolgus monkey and man, *Xenobiotica*, **1997**, *27*, 557–571.

Mibolerone



Molecular formula: $C_{20}H_{30}O_2$
Molecular weight: 302.46
CAS Registry No.: 3704-09-4
Merck Index: 6262
Lednicer No.: 2 144

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50

mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM

Retention time: 7.3
Limit of detection: 5 μg/mL

OTHER SUBSTANCES

Simultaneous: methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone
Interfering: dehydroepiandrosterone (UV 210)

KEY WORDS

oils; tablets; suspensions

REFERENCE

Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

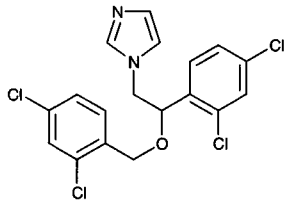
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide,

hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE
Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Miconazole



Molecular formula: $C_{18}H_{14}Cl_4N_2O$
Molecular weight: 416.13
CAS Registry No.: 22916-47-8, 22832-87-7 (nitrate)
Merck Index: 6266
Lednicer No.: 2 249

SAMPLE

Matrix: blood
Sample preparation: 400 μL Plasma + 400 μL water + 50 μL MeOH + 100 μL 1 M KOH + 6 mL hexane:dichloromethane 50:50, shake for 3 min, centrifuge at 4000 rpm for 6 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 80 × 4 CoPell ODS
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:10 mM pH 8.0 NaH_2PO_4 buffer 66:34
Flow rate: 2
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10
Internal standard: miconazole

OTHER SUBSTANCES

Extracted: sulconazole

KEY WORDS
plasma; dog; miconazole is IS

REFERENCE
Fass,M.; Zaro,B.; Chaplin,M.; Matin,S. Reversed-phase high-pressure liquid chromatographic analysis of sulconazole in plasma, *J.Pharm.Sci.*, **1981**, *70*, 1338–1340.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 202.8

CHROMATOGRAM
Retention time: 21.53

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Powders. Extract a sample equivalent to about 10 mg miconazole twice with 20 mL portions of MeOH with magnetic stirring, filter extracts, combine, dilute to 50 mL with MeOH. Remove a 2.5 mL aliquot and add it to 2 mL 200 μg/mL econazole in MeOH, dilute to 10 mL with MeOH, inject a 10 μL aliquot. Creams. Condition a Baker diol SPE cartridge with 6 mL dichloromethane. Add a sample equivalent to 10 mg miconazole to 30 mL dichloromethane, sonicate for 2 min, make up to 50 mL with dichloromethane, filter, add 2 mL of the filtrate to the SPE cartridge. Wash with two 3 mL portions of n-hexane:dichloromethane 4:1, aspirate to dryness, elute with three 1 mL portions of MeOH:100 mM triethylamine adjusted to pH 7.0 with acetic acid 4:1. Combine the eluates and dilute them to 5 mL with MeOH. Remove a 2.5 mL aliquot and add it to 1 mL 200 μg/mL econazole in MeOH, dilute to 5 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak RP-18
Mobile phase: MeOH:THF:100 mM triethylamine adjusted to pH 7.0 with acetic acid 70:12:18
Flow rate: 0.8
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 3.5
Internal standard: econazole (2.5)

KEY WORDS
creams; powders; SPE

REFERENCE
Cavrini,V.; Di Pietra,A.M.; Gatti,R. Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC), *J.Pharm.Biomed.Anal.*, **1989**, *7*, 1535–1543.

SAMPLE
Matrix: formulations
Sample preparation: Dilute peritoneal dialysis fluid with an equal volume of 4 μg/mL p-dichlorobenzene in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:50 mM pH 4.6 $(NH_4)H_2PO_4$ 85:15
Flow rate: 1
Injection volume: 20
Detector: UV 229

CHROMATOGRAM
Retention time: 8.5
Internal standard: p-dichlorobenzene (4.5)

KEY WORDS
stability-indicating; peritoneal dialysis fluid

REFERENCE
Holmes,S.E.; Aldous,S. Stability of miconazole in peritoneal dialysis fluid, *Am.J.Hosp.Pharm.*, **1991**, *48*, 286–290.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Powder tablets, weigh out amount equivalent to about 30 mg, add 100 mL MeOH, sonicate for 5 min, filter. Add a 2 mL aliquot of filtrate to 5 mL of 100 μg/mL ketoconazole in MeOH, make up to 25 mL with MeOH, inject 20 μL aliquot. Cream. Condition a 500 mg Bond-Elut diol cartridge with 6 mL dichloromethane. Weigh out cream equivalent to about 5 mg of drug, add 30 mL dichloromethane, sonicate for 3 min, make up to 100 mL with dichloromethane, filter. Add a 2 mL aliquot to the cartridge, wash with 2 mL dichloromethane:methanol 4:1, wash with 2 mL dichloromethane, elute with 3 mL MeOH:buffer 85:15. Add eluate to 0.5 mL 100 μg/mL ketoconazole in MeOH, make up to 5 mL with MeOH, inject 20 μL aliquot. (Buffer was 50 mM triethylamine adjusted to pH 7.0 with phosphoric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: THF:buffer 30:70 (Buffer was 50 mM triethylamine adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 18
Internal standard: ketoconazole (7)

OTHER SUBSTANCES
Simultaneous: clotrimazole, ketoconazole, bifonazole, tioconazole, isoconazole, econazole, fenticonazole

KEY WORDS
tablets; creams

REFERENCE
Di Pietra,A.M.; Cavrini,V.; Andrisano,V.; Gatti,R. HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 873–879.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, add metronidazole, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS C18
Mobile phase: MeCN:buffer 15:85 (Buffer was 10 mM NaH_2PO_4 adjusted to pH 8 with trimethylamine.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 3.93
Internal standard: metronidazole (2)

KEY WORDS
injections; stability-indicating; 5% dextrose; saline

REFERENCE
Faouzi,M.E.A.; Dine,T.; Luyckx,M.; Brunet,C.; Mallevais,M.-L.; Goudaliez,F.; Gressier,B.; Cazin,M.; Kablan,J.; Cazin,J.C. Stability, compatibility and plasticizer extraction of miconazole injection added to infusion solutions and stored in PVC containers, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1363–1372.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: RCSS Guard-Pak (Waters)
Column: 100 × 8 C18 Radial Pak (Waters)
Mobile phase: MeOH:0.75% acetic acid 30:70, pH adjusted to 5.5 with triethylamine
Flow rate: 3
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 14.1

OTHER SUBSTANCES
Simultaneous: acetaminophen, N-acetylprocainamide, cefaclor, cefamandole, cefazolin, cefotaxime, cefoxitin, cephalexin, cephalothin, cephapirin, chloramphenicol, cimetidine, moxalactam, procainamide, sulfamethoxazole, theophylline, tobramycin, vancomycin

REFERENCE
Danzer,L.A. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum, *Clin.Chem.*, **1983**, *29*, 856–858.

SAMPLE
Matrix: solutions
Sample preparation: Centrifuge at 10000 rpm, dilute the supernatant with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:50 mM $(NH_4)H_2PO_4$ 85:15
Detector: UV 230

REFERENCE
Okimoto,K.; Rajewski,R.A.; Uekama,K.; Jona,J.A.; Stella,V.J. The interaction of charged and uncharged drugs with neutral (HP-β-CD) and anionically charged (SBE7-β-CD) β-cyclodextrins, *Pharm.Res.*, **1996**, *13*, 256–264.

SAMPLE
Matrix: tissue
Sample preparation: Skin sample extracted with 500 μL mobile phase, vortex 1 min, centrifuge at 8000 rpm for 10 min, inject 40 μL aliquot.

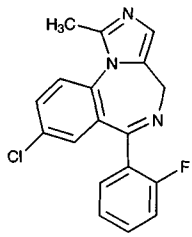
HPLC VARIABLES
Column: 125 × 4.5 Whatman 5 μm reverse-phase C18
Mobile phase: MeCN:10 mM KH_2PO_4 80:20
Flow rate: 0.7
Injection volume: 40
Detector: UV 214

CHROMATOGRAM
Retention time: 5.5
Limit of detection: 50 ng/mL

KEY WORDS
skin

REFERENCE
Pershing,L.K.; Corlett,J.; Jorgensen,C. In vivo pharmacokinetics and pharmacodynamics of topical ketoconazole and miconazole in human stratum corneum, *Antimicrob.Agents Chemother.*, **1994**, *38*, 90–95.

Midazolam



Molecular formula: $C_{18}H_{13}ClFN_3$
Molecular weight: 325.77
CAS Registry No.: 59467-70-8, 59467-96-8 (HCl), 59467-94-6 (maleate)
Merck Index: 6270
Lednicer No.: 3 197

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeOH + 500 μL 1 M NaOH + 3 mL hexane, shake for 5 min, centrifuge at 3000 rpm for 5 min. Evaporate 2 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm YMC-Guardpak ODS-AM (GL Sciences)
Column: 250 × 4.6 5 μm Inertsil ODS (GL Sciences)

Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 80:20
Flow rate: 1
Injection volume: 75
Detector: UV 245

CHROMATOGRAM
Limit of detection: 50 ng/mL (sic)

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL MeOH + 500 μL 1 M NaOH + 3 mL hexane, shake for 5 min, centrifuge at 3000 rpm for 5 min. Evaporate 2 mL of the organic phase to dryness under a stream of nitrogen. Dissolve residue in 200 μL mobile phase, inject a 75 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm YMC-Guardpack ODS-AM (GL Sciences, Japan)
Column: 250 × 4.6 5 μm Inertsil ODS
Mobile phase: MeCN:10 mM pH 6.5 phosphate buffer 8:2
Flow rate: 1
Injection volume: 75
Detector: UV 245

CHROMATOGRAM
Limit of detection: 50 ng/mL

KEY WORDS
plasma; rat; pharmacokinetics

REFERENCE
Takedomi,S.; Matsuo,H.; Yamano,K.; Yamamoto,K.; Iga,T.; Sawada,Y. Quantitative prediction of the interaction of midazolam and histamine H_2 receptor antagonists in rats, *Drug Metab.Dispos.*, **1998**, *26*, 318–323.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 1 M NaOH + 2 mL 10% (v/v) isopropanol in dichloromethane containing 25 ng/mL IS, vortex for 60 s, centrifuge at 2000 g for 45 s. Evaporate organic phase under a gentle stream of air at 60° for 10 min. Dissolve residue in 1 mL hexane:MTBE 2:1, add 200 μL 20 mM orthophosphoric acid, vortex for 30 s, centrifuge at 3200 g for 45 s. Carefully aspirate upper organic layer, add 7 μL 1 M NaOH to acidic aqueous phase to adjust pH to 6.6-6.9, inject an 80 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm C8 Symmetry (Waters)
Mobile phase: MeCN:THF:10 mM pH 6.7 phosphate buffer 35:5:60
Flow rate: 1
Injection volume: 80
Detector: UV 220

CHROMATOGRAM
Retention time: 7.4
Internal standard: climazolam (8.4)
Limit of detection: 10 ng/mL
Limit of quantitation: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Noninterfering: amoxicillin, caffeine, carbamazepine, cisapride, dexamethasone, dopamine, furosemide, gentamicin, indomethacin, morphine, phenobarbital, ranitidine, theophylline

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Lee,T.C.; Charles,B. Measurement by HPLC of midazolam and its major metabolite, 1-hydroxymidazolam in plasma of very premature neonates, *Biomed.Chromatogr.*, **1996**, *10*, 65–68.

SAMPLE
Matrix: blood
Sample preparation: Add 25 μL 40 μg/mL diazepam in MeOH, 40 μL 2% NaOH, and 3.5 mL cyclohexane:diethyl ether 31:69 to 1 mL plasma. Extract on a rotary mixer at 4° for 10 min, centrifuge at 4° at 2000 g for 10 min. Remove a 3.3 mL aliquot of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°. Dissolve the residue in 300 μL MeCN: water 5:95, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 0.8 μ-Precolumn cartridge C18 (LC Packings)
Column: 150 × 0.8 3 μm Hypersil C18 BDS
Mobile phase: Gradient. MeCN:10 mM pH 7.0 sodium phosphate buffer 35:65 for 16 min, to 60: 40 over 1 min, maintain at 60:40.
Flow rate: 0.016
Injection volume: 20
Detector: UV 240 for 17.6 min then UV 300

CHROMATOGRAM
Retention time: 15.4
Internal standard: diazepam (19.5)
Limit of quantitation: 1 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, 1'-hydroxymidazolam

KEY WORDS
pharmacokinetics; capillary HPLC; plasma

REFERENCE
Eeckhoudt,S.L.; Desager,J.-P.; Horsmans,Y.; De Winne,A.J.; Verbeeck,R.K. Sensitive assay for midazolam and its metabolite 1'-hydroxymidazolam in human plasma by capillary high-performance liquid chromatography, *J.Chromatogr.B*, **1998**, *710*, 165–171.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 1 M pH 9.0 borate buffer to 50 μL serum, mix well, add 1 mL chloroform:diethyl ether 95:5 (Caution! Chloroform is a carcinogen!), vortex for 1 min, centrifuge at 1100 for 5 min, evaporate the organic layer to dryness under nitrogen at 40°, resuspend the residue in 50 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 2.1 C18 symmetry column (Waters)
Mobile phase: MeCN:MeOH:14.9 mM pH 3 sodium acetate 23:10:67
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 4.7
Limit of detection: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
pharmacokinetics; rat; serum

REFERENCE
Ma,F.; Lau,C.E. Determination of midazolam and its metabolites in serum microsamples by high-performance liquid chromatography and its application to pharmacokinetics in rats, *J.Chromatogr.B*, **1996**, *682*, 109–113.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 14.873

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool microsomal incubation on ice, add 100 μL MeCN and IS, centrifuge, inject an aliquot of the supernatant.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer 22.5:37.5:40
Flow rate: 0.8
Detector: UV 220

CHROMATOGRAM
Retention time: 14.1
Internal standard: phenacetin (5.4)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Schmider,J.; Duan,S.X.; Wright,C.E.; Harmatz,J.S.; Shader,R.I. Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents, *J.Clin.Pharmacol.*, **1996**, *36*, 783–791.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Cool incubation mixture on ice, add 100 μL MeCN and IS, centrifuge, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:10 mM phosphate buffer 22.5:37.5:40
Flow rate: 0.8
Detector: UV 220

CHROMATOGRAM
Retention time: 14.2
Internal standard: phenacetin (5.4)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
liver

REFERENCE
von Moltke,L.L.; Greenblatt,D.J.; Schmider,J.; Duan,S.X.; Wright,C.E.; Harmatz,J.S.; Shader,R.I. Midazolam hydroxylation by human liver microsomes in vitro: Inhibition by fluoxetine, norfluoxetine, and by azole antifungal agents, *J.Clin.Pharmacol.*, **1996**, *36*, 783–791.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 2 mg/mL solution in 0.9% sodium chloride, dilute 1:1000 with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: Bakerbond phenyl column
Mobile phase: MeCN:20 mM sodium dihydrogen phosphate 80:20
Flow rate: 2
Detector: UV 240

CHROMATOGRAM
Retention time: 9.2

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
stability-indicating

REFERENCE
Stiles,M.L.; Allen,L.V.,Jr.; Prince,S.J. Stability of deferoxamine mesylate, floxuridine, fluorouracil, hydromorphone hydrochloride, lorazepam, and midazolam hydrochloride in polypropylene infusion-pump syringes, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 1583–1588.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize brain in 100 mM NaOH (5 mL/g) by sonication. Add 60 μL 1 μg/mL IS in MeOH to a tube, dry in a water bath under a stream of nitrogen at 50°. Add 250 μL homogenate (ca. 50 mg tissue equivalent) and 250 μL 100 mM pH 13 NaOH, vortex thoroughly. Add 2 mL toluene, mix (50 inversions), centrifuge at 15 000 rpm at 4° for 20 min. Dry the organic phase under a stream of nitrogen. Add 2 mL toluene to the aqueous phase and repeat the extraction. Combine the organic layers and dry under a stream of nitrogen. Reconstitute the residue in 100 μL mobile phase, inject a 40 μL aliquot.

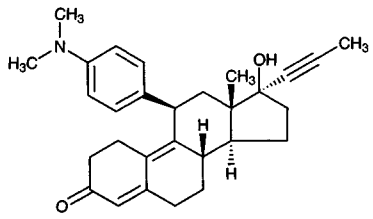
HPLC VARIABLES
Column: 100 × 4.6 3 μm Rainin C8 Microsorb
Mobile phase: MeCN:MeOH:25 mM potassium phosphate buffer 18.5:16.5:65
Flow rate: 0.9
Injection volume: 40
Detector: UV 240

CHROMATOGRAM
Retention time: 4.2
Internal standard: halazepam (6.2)
Limit of quantitation: 500 ng/g

KEY WORDS
brain; rat

REFERENCE
Jiang,Q.; Walton,N.Y.; Gunawan,S.; Treiman,D.M. High-performance liquid chromatographic determination of midazolam in rat brain, *J.Chromatogr.B*, **1996**, *683*, 276–280.

Mifepristone



Molecular formula: $C_{29}H_{35}NO_2$
Molecular weight: 429.60
CAS Registry No.: 84371-65-3
Merck Index: 6273
Lednicer No.: 5 53

SAMPLE
Matrix: blood
Sample preparation: Add 200-400 μL plasma or serum to a Pasteur pipette packed with 3 mL 60-80 mesh Chromosorb W-NAW:ethylene glycol 80:20 (w/w), let stand for 30 min, elute with 5 mL n-hexane:ethyl acetate 95:5. Evaporate the eluate and reconstitute the residue in mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: Lichrosorb RP-18
Mobile phase: MeOH:water:triethanolamine 90:10:0.05
Flow rate: 1.5
Injection volume: 100
Detector: UV 304

CHROMATOGRAM
Retention time: 4.33
Limit of detection: 4 ng

KEY WORDS
plasma; serum; SPE; pharmacokinetics

REFERENCE
Heikinheimo,O.; Tevilin,M.; Shoupe,D.; Croxatto,H.; Lahteenmaki,P. Quantitation of RU 486 in human plasma by HPLC and RIA after column chromatography, *Contraception*, **1986**, *34*, 613–624.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 12.6 μg/mL IS in MeOH + 650 μL water, mix, inject a 400 μL aliquot into column A and elute to waste with mobile phase A for 7 min, elute the contents of column A onto column B with mobile phase B, after 10 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Wash column A with MeOH:water 70:30 for 5 min then re-equilibrate with water.

HPLC VARIABLES
Column: A 30 × 4 Serumout (Sekisui); B 300 × 3.9 μBondapak C18
Mobile phase: A water; B MeCN:MeOH:50 mM pH 3.1 phosphate buffer 27:18:60
Column temperature: 25
Flow rate: A 0.6; B 1
Injection volume: 400
Detector: UV 305 or E, Sekisui ECD-120, +1.0 V, Ag/AgCl reference electrode

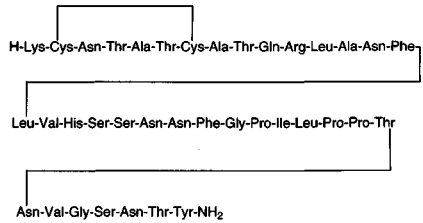
CHROMATOGRAM
Retention time: 30.1
Internal standard: 13-ethyl-17-hydroxy-18,19-dinorpregna-4,9,11-trien-20-yn-3-one (R2323) (35.3)
Limit of detection: 0.9 ng (E), 6.6 ng (UV)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
serum; pharmacokinetics; column-switching

REFERENCE
Nagoshi,K.; Hayashi,N.; Sekiba,K. Automated direct assay system for RU38486, an antiprogesterone-antiglucocorticoid agent, and its metabolites using high performance liquid chromatography, *Acta Med.Okayama.*, **1991**, *45*, 81–87.

Miloxacin



Molecular formula: $C_{12}H_9NO_6$
Molecular weight: 263.21
CAS Registry No.: 37065-29-5
Merck Index: 6283

SAMPLE
Matrix: blood, tissue
Sample preparation: Blood. Filter serum through a 0.45 μm syringe filter with a cellulose acetate membrane, inject a 50 μL aliquot of the filtrate. Tissue. Add 1 mL MeCN:THF 95:5 to 1 g muscle, homogenize with a Pencil Mixer (Iuchi, Japan) for 2 min, centrifuge at 1500 g for 5 min, filter the supernatant through a syringe filter unit, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μL Hisep shielded hydrophobic phase precolumn (Supelco)
Column: 150 × 4.6 5 μL Hisep shielded hydrophobic phase (Supelco)
Mobile phase: MeCN:buffer 15:85 (Buffer was 50 mM citric acid:200 mM pH 2.5 Na_2HPO_4 buffer containing 10 mM tetra-*n*-butyl ammonium bromide 85:15.)
Flow rate: 1

Injection volume: 20-50
Detector: UV 265

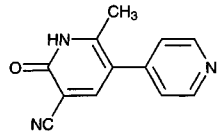
CHROMATOGRAM
Retention time: 11.5
Limit of detection: 50 ng/mL (serum), 100 ng/mL (muscle)

OTHER SUBSTANCES
Extracted: sulfamonomethoxine, oxolinic acid

KEY WORDS
fish; muscle; serum

REFERENCE
Ueno,R.; Aoki,T. High-performance liquid chromatographic method for the rapid and simultaneous determination of sulfamonomethoxine, miloxacin and oxolinic acid in serum and muscle of cultured fish, *J.Chromatogr.B*, **1996**, *682*, 179–181.

Milrinone



Molecular formula: $C_{12}H_9N_3O$
Molecular weight: 211.22
CAS Registry No.: 78415-72-2
Merck Index: 6284

SAMPLE
Matrix: blood, tissue
Sample preparation: Homogenize (Ystral D7801) tissue with 2 volumes of 10 mM sodium bicarbonate containing 2.5% acetic acid (final pH 3), centrifuge at 20000 g for 30 min. 100 μL Plasma or tissue homogenate + 375 μL water, mix, add 25 μL 50% acetic acid, vortex, centrifuge at 7000 g for 5 min. Filter (Amicon Centricon-30 microconcentrator) while centrifuging in a refrigerated centrifuge at 5500 g for 35 min, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 3 RP-2 (Chrompack)
Column: 250 × 4 RP Select B RT (Merck)
Mobile phase: MeOH:25 mM pH 3.75 ammonium acetate containing 0.01% tetramethylammonium hydroxide
Flow rate: 0.75
Injection volume: 100
Detector: UV 331

CHROMATOGRAM
Retention time: 5
Limit of detection: 5 ng/mL

KEY WORDS
rat; liver; heart; brain; lung; plasma

REFERENCE
Verrijk,R.; van Rooij,H.H.; Wemer,J.; Porsius,A.J. High-performance liquid chromatographic determination of milrinone in biological tissues and fluids, *J.Chromatogr.*, **1989**, *491*, 265–268.

SAMPLE
Matrix: blood, urine
Sample preparation: Urine. Acidify urine to pH 3.75 with 50% acetic acid, filter (Amicon Centricon-30 microconcentrator), inject an aliquot. Plasma. 100 μL Plasma + 375 μL water, mix,

add 25 μL 50% acetic acid, vortex, centrifuge at 7000 g. Filter (Amicon Centricon-30 microconcentrator) while centrifuging in a refrigerated centrifuge at 5500 g for 35 min, inject an aliquot of the filtrate.

HPLC VARIABLES
Guard column: 10 × 3 RP-2 (Chrompack)
Column: 250 × 4 RP Select B RT (Merck)
Mobile phase: MeOH:25 mM pH 3.75 ammonium acetate containing 0.01% tetramethylammonium hydroxide
Flow rate: 0.75
Injection volume: 100
Detector: UV 331

CHROMATOGRAM
Limit of detection: 5 ng/mL

KEY WORDS
rat; plasma; pharmacokinetics

REFERENCE
Verrijk,R.; Vleeming,W.; van Rooij,H.H.; Wemer,J.; Porsius,A.J. Plasma elimination of milrinone in rats in relation to its hemodynamic effects, *J.Pharm.Sci.*, **1990**, *79*, 236–239.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Sample + 99 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS III
Mobile phase: MeOH:500 mM pH 7 borate buffer:water 40:2:58
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 2.5

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: digoxin

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Riley,C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Sample + 9 mL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb phenyl
Mobile phase: MeCN:500 mM KH_2PO_4:water 22:10:68 adjusted to pH 7.1 with 10 M NaOH
Flow rate: 2
Injection volume: 20
Detector: UV 268

CHROMATOGRAM
Retention time: 4

OTHER SUBSTANCES
Simultaneous: procainamide, degradation products

KEY WORDS
stability-indicating; 5% dextrose; injections

REFERENCE
Riley,C.M. Stability of milrinone and digoxin, furosemide, procainamide hydrochloride, propranolol hydrochloride, quinidine gluconate, or verapamil hydrochloride in 5% dextrose injection, *Am.J.Hosp.Pharm.*, **1988**, *45*, 2079–2091.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with water, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Whatman PXS ODS-3 C18
Mobile phase: MeCN:Buffer 25:75 (Buffer is 1.08 g sodium octanesulfonate in 900 mL of water adjusted to pH 3.5 with glacial acetic acid and diluted to 1 L with water.)
Flow rate: 2
Injection volume: 20
Detector: UV 229

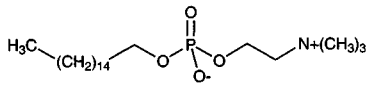
CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: atropine

KEY WORDS
injections; 10% calcium chloride; 7.5% sodium bicarbonate; stability-indicating

REFERENCE
Wilson,T.D.; Forde,M.D. Stability of milrinone and epinephrine, atropine sulfate, lidocaine hydrochloride, or morphine sulfate injection, *Am.J.Hosp.Pharm.*, **1990**, *47*, 2504–2507.

Miltefosine



Molecular formula: $C_{21}H_{46}NO_4P$
Molecular weight: 407.57
CAS Registry No.: 58066-85-6
Merck Index: 6285

SAMPLE
Matrix: blood
Sample preparation: Lyophilize 1 mL serum, extract residue 3 times with 1 mL portions of MeOH. Combine the extracts, add 500 μL of a saturated solution of potassium tert-butoxide in MeOH, heat at 50° for 15 min, add 1 mL 3 M HCl, heat at 50° for 15 min, neutralize with 250 μL 2 M NaOH, wash twice with 2 mL portions of n-hexane. Extract the MeOH/water phase with chloroform. Evaporate the chloroform layer to dryness under a stream of nitrogen, reconstitute the residue in 500 μL MeCN:MeOH:water 20:57:23, inject an aliquot.

HPLC VARIABLES
Guard column: 70 × 3.2 30-40 μm SC-201 RP (Vydac)

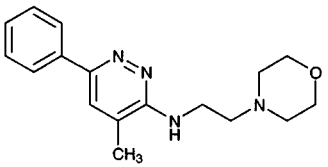
Column: 250 × 4.6 5 μm Nucleosil 120-5 C18
Mobile phase: MeCN:MeOH:water 20:57:23 containing 20 mM choline chloride
Flow rate: 2
Detector: UV 203 or radioactivity

CHROMATOGRAM
Retention time: 22

KEY WORDS
rat; serum; pharmacokinetics; tritium-labeled

REFERENCE
Unger,C.; Fleer,E.; Damenz,W.; Hilgard,P.; Nagel,G.; Eibl,H. Hexadecylphosphocholine: determination of serum concentrations in rats, *J.Lipid Mediat.*, **1991**, *3*, 71–78.

Minaprine



Molecular formula: $C_{17}H_{22}N_4O$
Molecular weight: 298.39
CAS Registry No.: 25905-77-5, 25953-17-7 (2.HCl)
Merck Index: 6287
Lednicer No.: 4 120

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

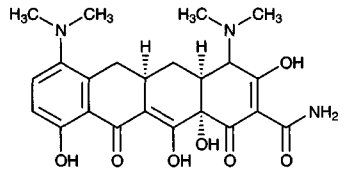
HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 204

CHROMATOGRAM
Retention time: 11.225

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Minocycline



Molecular formula: $C_{23}H_{27}N_3O_7$
Molecular weight: 457.48
CAS Registry No.: 10118-90-8, 13614-98-7 (HCl)
Merck Index: 6289
Lednicer No.: 1 214

SAMPLE

Matrix: blood
Sample preparation: Mix 1 mL plasma with 400 μL 500 mM pH 5 KH_2PO_4 and 5 mL ethyl acetate, shake for 1 min, centrifuge at ≥2000 g for 2 min, mix 4 mL of the upper organic phase with 500 μL 20 mM HCl, shake for 1 min, centrifuge at ≥2000 g for 2 min, inject a 20 μL aliquot of the lower aqueous phase.

HPLC VARIABLES

Column: 125 × 4.0 Nucleosil 5-CN
Mobile phase: MeOH:20 mM perchloric acid containing 4 mM triethylamine 20:80
Flow rate: 1
Detector: UV 350

CHROMATOGRAM

Retention time: 2.8
Limit of quantitation: 30 ng/mL

KEY WORDS

plasma

REFERENCE

Mascher,H.J. Determination of minocycline in human plasma by high-performance liquid chromatography with UV detection after liquid-liquid extraction, *J.Chromatogr.A*, **1998**, *812*, 339–342.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 20 μL trifluoroacetic acid, mix 30 s in a whirl mixer, centrifuge at 5400 g for 5 min, inject supernatant (80 μL).

HPLC VARIABLES

Guard column: 10 μm Waters RP phenyl
Column: 150 × 3.9 10 μm Waters RP phenyl
Mobile phase: Gradient. A 0.1 M diammonium EDTA:1 M diethanolamine (to pH 7.3 with 85% phosphoric acid):isopropanol:water 10:50:185:755. B 0.1 M diammonium EDTA:1 M diethanolamine (to pH 7.3 with 85% phosphoric acid):isopropanol:water 10:50:400:540. From 100% A to 100% B over 6 min
Flow rate: 2
Injection volume: 80
Detector: UV 340

CHROMATOGRAM

Retention time: 4.1
Limit of detection: 35 ng/mL

KEY WORDS

plasma

REFERENCE

Krämer-Horaczynska,F. High-performance liquid chromatographic procedures for the quantitative analysis of 15 tetracycline derivatives in small blood samples, *J.Chromatogr.Sci.*, **1991**, *29*, 107–113.

SAMPLE
Matrix: blood
Sample preparation: 200 μL Serum + 50 μL 20 μg/mL oxytetracycline in water + 250 μL 500 mM trichloroacetic acid, vortex for 30 s, centrifuge at 10000 g for 10 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: Nova-Pak phenyl guard
Column: 4 μm Nova-Pak phenyl radial compression
Mobile phase: MeCN:MeOH:100 mM oxalic acid 11:9:80 adjusted to pH 2.7 with 1 M HCl
Flow rate: 2
Injection volume: 50
Detector: UV 350

CHROMATOGRAM
Retention time: 8-9
Internal standard: oxytetracycline (13.5-14)
Limit of quantitation: 620 ng/mL

KEY WORDS
serum; pharmacokinetics

REFERENCE
Birmingham,K.; Vaughan,L.M.; Strange,C. Rapid serum minocycline assay for pleurodesis monitoring using high-performance liquid chromatography with radial compression, *Ther.Drug Monit.*, **1995**, *17*, 268–272.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 22.637

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, formulations

Sample preparation: Bulk. Dissolve 25 mg minocycline hydrochloride in solvent, make up to 25 mL with solvent, inject an aliquot. Capsules, tablets. Add an amount equivalent to 25 mg minocycline hydrochloride to 25 mL solvent, sonicate for 5 min, centrifuge at 2500 g for 5 min, filter (1.5 μm), inject an aliquot of the filtrate. (Solvent was 10 mM NaOH containing 0.1% sodium sulfite.)

HPLC VARIABLES
Column: 250 × 4.6 8 μm 100 Å PLRP-S poly(styrene-divinylbenzene) (Polymer Labs)
Mobile phase: t-Butanol:200 mM pH 10.5 phosphate buffer:TBAS solution:EDTA solution:water 9:10:10:10:61 (TBAS solution was 20 mM tetrabutylammonium sulfate adjusted to pH 10.5 with NaOH solution. EDTA solution was 10 mM EDTA adjusted to pH 10.5 with NaOH solution.)
Column temperature: 60
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
capsules; tablets

REFERENCE
Weng,N.; Thuranira,J.; Vermeulen,K.; Roets,E.; Hoogmartens,J. Quantitative analysis of minocycline by liquid chromatography on poly(styrene-divinylbenzene), *J.Liq.Chromatogr.*, **1992**, *15*, 2529–2549.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 10-100 μg/mL solution in buffer, inject an aliquot. Capsules, tablets. Prepare a 1 mg/mL solution of capsule contents or crushed tablets in buffer, sonicate for 10 min, filter (0.45 μm), dilute with buffer, inject an aliquot. (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm 100 Å PLRP-S polystyrene-divinylbenzene (Polymer Laboratories)
Mobile phase: MeCN:buffer 20:80 (Buffer was 20 mM sodium perchlorate adjusted to pH 2.0 with perchloric acid.)
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
capsules; tablets

REFERENCE
Bryan,P.D.; Stewart,J.T. Chromatographic analysis of selected tetracyclines from dosage forms and bulk drug substance using polymeric columns with acidic mobile phases, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 675–692.

SAMPLE
Matrix: cells
Sample preparation: 100 μL Cell suspension + 100 μL cefoperazone solution + 100 μL Hanks balanced salt solution, sonicate 30 min, add 800 μL MeCN, centrifuge at 13000 g for 5 min,

remove supernatant. Dry supernatant under air, dissolve in 100 μL mobile phase, inject 75 μL.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:50 mM pH 3.1 KH_2PO_4 45:55
Flow rate: 1
Injection volume: 75
Detector: UV 353

CHROMATOGRAM
Retention time: 3
Internal standard: tetracycline
Limit of detection: 100-1000 ng/mL

REFERENCE
Darouiche,R.O.; Hamill,R.J. Antibiotic penetration of and bactericidal activity within endothelial cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1059–1064.

SAMPLE
Matrix: formulations
Sample preparation: Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Vydac 208TP54 C8
Mobile phase: MeCN:buffer 22:78 (Buffer was 50 mM K_2HPO_4 adjusted to pH 6.5 with phosphoric acid.)
Flow rate: 1
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2

OTHER SUBSTANCES
Simultaneous: degradation products
Noninterfering: rifampin

KEY WORDS
injections; saline; 5% dextrose; stability-indicating

REFERENCE
Pearson,S.D.; Trissel,L.A. Stability and compatibility of minocycline hydrochloride and rifampin in intravenous solutions at various temperatures, *Am.J.Hosp.Pharm.*, **1993**, *50*, 698–702.

SAMPLE
Matrix: honey
Sample preparation: Condition a 500 mg Baker-10 C18 SPE cartridge with 10 mL MeOH, 10 mL water, and 10 mL saturated aqueous disodium EDTA. Condition a 500 mg Baker-10 COOH cartridge with MeOH:ethyl acetate 10:90. Dissolve 25 g honey in 50 mL 100 mM pH 4.0 disodium EDTA-McIlvaine buffer, filter. Add the filtrate to the C18 SPE cartridge, wash with 20 mL water, wash with 400 μL ethyl acetate, air dry under vacuum for 5 min, elute with 50 mL MeOH:ethyl acetate 10:90. Add a 5 mL aliquot to the COOH SPE cartridge, wash with 5 mL MeOH (?), elute with 10 mL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Chemcosorb 3C8 (Chemco)
Mobile phase: MeCN:MeOH:10 mM aqueous oxalic acid 3:2:16, pH 3.0
Flow rate: 1
Injection volume: 100
Detector: UV 350

CHROMATOGRAM
Retention time: 2
Limit of detection: 0.1 ppm

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline (demethylchlortetracycline), doxycycline, methacycline, oxytetracycline, tetracycline

KEY WORDS
SPE

REFERENCE
Oka,H.; Ikai,Y.; Kawamura,N.; Uno,K.; Yamada,M.; Harada,K.; Suzuki,M. Improvement of chemical analysis of antibiotics. XII. Simultaneous analysis of seven tetracyclines in honey, *J.Chromatogr.*, **1987**, *400*, 253–261.

SAMPLE
Matrix: milk
Sample preparation: Fill a disposable polypropylene column (Bio-Rad Econo-Pac column) with Chelating Sepharose Fast Flow (Pharmacia) and condition it with 10 mL water, 1.5 mL 100 mM copper sulfate, and 100 mL water. Condition a 6 mL SupelClean ENVI-Chrom P SPE cartridge with 2 mL MeOH and 5 mL water. Homogenize 10 g tissue with 20-30 mL 100 mM pH 4 succinic acid buffer. Centrifuge the homogenate at 2000 g at 10° for 15-20 min. Add the supernatant to the metal chelate affinity column, wash sequentially with 5 mL 500 mM NaCl, 10 mL water, 10 mL MeOH, 10 mL water, and 3 mL McIlvaine buffer, discard the clear effluent. Elute with 8 mL McIlvaine-EDTA-NaCl buffer. Add the eluate to the SPE cartridge under gravity, rinse the column with 2.5 mL water, add the rinse to the SPE cartridge. Wash the SPE cartridge with 2.5 mL water. Dry the SPE cartridge by drawing air through it for 2-3 min. Elute with 5 mL MeOH. Evaporate the eluate to dryness under nitrogen at 40-50°, dissolve the residue in 1 mL water. Inject a 100 μL aliquot. (McIlvaine buffer was 500 mM NaCl and 100 mM EDTA (Carson, M.C. J. AOAC Int. 1993, 76, 329).)

HPLC VARIABLES
Column: 150 × 3.9 5 μm PLRP-S (Polymer Labs, USA)
Mobile phase: MeOH:5 mM oxalic acid 58:42
Flow rate: 0.5
Injection volume: 100
Detector: MS, HP 5989, NICI, high energy dynode, HP 59980B particle beam interface 60°, helium sheath 40-45 p.s.i., source 250°, quadrupole 100°, source pressure 1 Torr with methane reagent gas, m/z 378-483

CHROMATOGRAM
Retention time: 6.75

OTHER SUBSTANCES
Extracted: chlortetracycline, demeclocycline, doxycycline, oxytetracycline, tetracycline

KEY WORDS
metal chelate affinity chromatography; cow; SPE

REFERENCE
Carson,M.C.; Ngoh,M.A.; Hadley,S.W. Confirmation of multiple tetracycline residues in milk and oxytetracycline in shrimp by liquid chromatography-particle beam mass spectrometry, *J.Chromatogr.B*, **1998**, *712*, 113–128.

SAMPLE
Matrix: milk
Sample preparation: Prepare a column as follows. Swirl Chelating Sepharose Fast Flow resin (Pharmacia) in its bottle, add it to a polypropylene column to give a bed volume of 1.0-1.2 mL, wash 3 times with 2 mL portions of water, wash with 2 mL 10 mM copper sulfate, wash with two 2 mL portions of water. Centrifuge 5 mL milk at 10° at 1500 g for 15 min, remove the

lower layer and add it to 10 mL succinate buffer, mix, centrifuge at 1500 g for 30 min, add the supernatant to the column. Wash with 2 mL succinate buffer, wash with 2 mL water, wash with 2 mL MeOH, wash with 2 mL water, wash with 700 μL citrate/phosphate buffer (be careful not to disturb bed), elute with 2.5 mL citrate/phosphate buffer (column is white and eluate is blue). Filter (Amicon Centricon 30, MW 30000 cut-off; pre-washed by centrifuging with 2 mL water) while centrifuging at 5000 g for 30-90 min, inject a 600 μL aliquot of the ultrafiltrate. (Prepare succinate buffer by dissolving 11.8 g succinic acid in 980 mL water, adjust pH to 4.0 with 10 M NaOH, make up to 1 L. Prepare the citrate/phosphate buffer by dissolving 12.9 g citric acid monohydrate, 10.9 g Na_2HPO_4, 37.2 g disodium EDTA dihydrate, and 29.2 g NaCl in 1 L water.)

HPLC VARIABLES

Column: 150 × 4.6 5 μm PLRP-S (Polymer Labs)
Mobile phase: Gradient. MeCN:MeOH:10 mM oxalic acid 0:0:100 for 1 min, to 22:8:70 over 5 min, maintain at 22:8:70 for 11 min, return to initial conditions.
Flow rate: 1
Injection volume: 600
Detector: UV 355

CHROMATOGRAM

Retention time: 11.7
Limit of detection: 0.50 ng/mL
Limit of quantitation: 1.03 ng/mL

OTHER SUBSTANCES

Extracted: chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, tetracycline
Noninterfering: chloramphenicol, gentian violet, hydromycin B, ivermectin, spectinomycin, sulfa drugs

KEY WORDS

cow; SPE; ultrafiltrate

REFERENCE

Carson,M.C. Simultaneous determination of multiple tetracycline residues in milk using metal chelate affinity chromatography, *J.AOAC Int.*, **1993**, *76*, 329–334.

SAMPLE

Matrix: urine
Sample preparation: Adjust the pH of 80 mL urine to 6.5 by adding 5.6 g NaH_2PO_4 and 10 g sodium sulfite, extract with 100 mL ethyl acetate. Remove the organic layer and evaporate it to dryness under reduced pressure at room temperature, reconstitute the residue in 1 mL mobile phase, inject a 20-100 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 5 μm Nucleosil SA (strong cation-exchange)
Mobile phase: EtOH:buffer 48:52 (Buffer was 100 mM pH 4.6 citrate containing 0.05% disodium EDTA.)
Flow rate: 1
Injection volume: 20-100
Detector: UV 350

CHROMATOGRAM

Retention time: 5

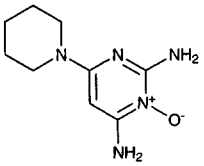
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Nelis,H.J.C.F.; De Leenheer,A.P. Metabolism of minocycline in humans, *Drug Metab.Dispos.*, **1982**, *10*, 142–146.

Minoxidil



Molecular formula: $C_9H_{15}N_5O$
Molecular weight: 209.25
CAS Registry No.: 38304-91-5
Merck Index: 6290
Lednicer No.: 1 262

SAMPLE

Matrix: blood
Sample preparation: Condition a Fisher Prepsep C18 SPE column with 2 volumes MeOH then 2 volumes water. 1 mL Plasma + 50 μL 0.056 μg/mL IS in water, vortex for 15 s, add to SPE column, wash with 2 volumes water, wash with 6 mL acetone, elute with ten 200 μL fractions of MeOH, dry at 50° under a stream of nitrogen, reconstitute in 100 μL mobile phase, vortex for 10 s, inject a 10-60 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:water 11:98, adjusted to pH 3.0 with phosphoric acid
Flow rate: 1.5
Injection volume: 10-60
Detector: E, Environmental Sciences Assoc. Model 5100 A, guard cell ESA Model 5020 +0.90 V, detector ESA model 5011 with detector 1 +0.30 V and detector 2 +0.80 V (UV 280 J.Pharm.Sci.1990, 79, 483)

CHROMATOGRAM

Retention time: 12 (minoxidil), 18 (minoxidil sulfate)
Internal standard: 2,4-diamino-6-diethylaminopyrimidine 3-oxide (10)
Limit of detection: 500 pg/mL

KEY WORDS

plasma; SPE

REFERENCE

Carrum,G.; Abernethy,D.R.; Sadhukhan,M.; Wright,C.E. Minoxidil analysis in human plasma using high-performance liquid chromatography with electrochemical detection. Application to pharmacokinetic studies, *J.Chromatogr.*, **1986**, *381*, 127–135.

SAMPLE

Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES

Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 231

CHROMATOGRAM

Retention time: 4.01

Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromethazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18

Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30

Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)

Injection volume: 10-30
Detector: UV 231.1

CHROMATOGRAM
Retention time: 9.76

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve a sample in MeOH to a concentration of about 1 mg/mL, inject an aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Spherisorb SCX
Mobile phase: MeOH:water 80:20 containing 20 mM ammonium formate and 2.3 mL/L trifluoroacetic acid
Flow rate: 1
Injection volume: 1-10
Detector: UV 270

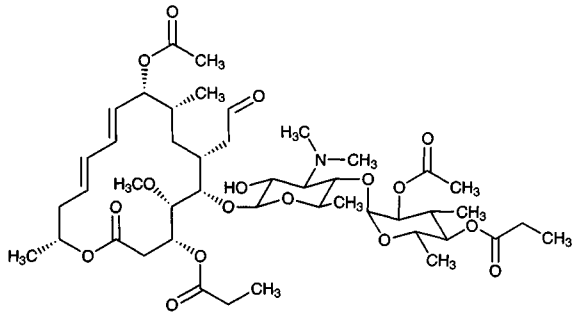
CHROMATOGRAM
Retention time: 4.7

OTHER SUBSTANCES
Simultaneous: cimetidine, clomipramine, halofantrine, haloperidol, reserpine, verapamil

REFERENCE
Law,N.; Appleby,J.R.G. Re-evaluation of strong cation-exchange high-performance liquid chromatography for the analysis of basic drugs, *J.Chromatogr.A*, **1996**, *725*, 335–341.

Miokamycin

Molecular formula: $C_{45}H_{71}NO_{17}$
Molecular weight: 898.06
CAS Registry No.: 55881-07-7
Merck Index: 6291



SAMPLE
Matrix: bulk
Sample preparation: Dissolve 60 mg of bulk compound in 50 mL MeCN, add 5 mL 1.2 mg/mL josamycin in MeCN, mix, inject an aliquot.

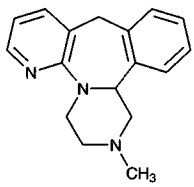
HPLC VARIABLES
Column: 150 × 2.6 5 μm Spheri ODS-2

Mobile phase: MeCN:buffer 75:25 (Buffer was 10 mM ammonium acetate containing 1 mM K_2HPO_4 adjusted to pH 6.5 with 10 mM phosphoric acid.)
Column temperature: 25
Flow rate: 1.5
Detector: UV 232

CHROMATOGRAM
Retention time: 7.0
Internal standard: josamycin (3.2)

REFERENCE
Marini,D.; Balestrieri,F.; Pollino,G. Dosaggio mediante HPLC della miocamicina e delle sue principali impurezze [HPLC analysis of miokamycin and its principle impurities], *Boll.Chim.Farm.*, **1986**, *125*, 193–196.

Mirtazepine



Molecular formula: $C_{17}H_{19}N_3$
Molecular weight: 265.36
CAS Registry No.: 61337-67-5
Merck Index: 6295
Lednicer No.: 5 177

SAMPLE
Matrix: formulations
Sample preparation: Extract 300 mg powdered tablet with 100 mL MeCN:water 50:50, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:THF:buffer 14.875:12.67:7.455:65 (Buffer was 18 g/L tetramethylammonium hydroxide pentahydrate in water adjusted to pH 7.4 with 85% phosphoric acid.)
Column temperature: 40
Flow rate: 1.5
Injection volume: 10
Detector: UV 210

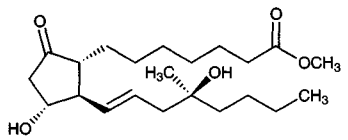
CHROMATOGRAM
Limit of detection: 0.01-0.04%
Limit of quantitation: 0.02-0.12%

OTHER SUBSTANCES
Simultaneous: impurities

KEY WORDS
tablets; comparison with capillary electrophoresis

REFERENCE
Wynia,G.S.; Windhorst,G.; Post,P.C.; Maris,F.A. Development and validation of a capillary electrophoresis method within a pharmaceutical quality control environment and comparison with high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *773*, 339–350.

Misoprostol



Molecular formula: $C_{22}H_{38}O_5$
Molecular weight: 382.54
CAS Registry No.: 59122-46-2
Merck Index: 6297

SAMPLE

Matrix: formulations
Sample preparation: Extract polymeric controlled-release formulations under supercritical fluid conditions using carbon dioxide:formic acid 95:5 at 330 atmospheres at 75° in a 500 μL cell, restrictor temperature 100°, collection solvent 15 mL hexane:EtOH 2:1, collection solvent temperature 0°, extraction time 1 h. Evaporate the collection solvent to dryness, reconstitute with 1 mL mobile phase, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Supelco ODS
Mobile phase: MeCN:MeOH:water 45:20:35
Flow rate: 1.5
Injection volume: 10
Detector: UV 280 following post-column reaction. The column effluent mixed with 4 M KOH pumped at 0.5 mL/min and the mixture flowed at 80° to the detector.

CHROMATOGRAM

Retention time: 7.5
Limit of detection: <0.1 ppt

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

polymeric controlled-release formulations; SFE; post-column reaction

REFERENCE

Roston,D.A.; Sun,J.J.; Collins,P.W.; Perkins,W.E.; Tremont,S.J. Supercritical fluid extraction-liquid chromatography method development for a polymeric controlled-release drug formulation, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1513–1520.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH:water 60:40, inject a 20 μL aliquot onto column A with mobile phase A. When the 11R,16S and 11S,16R enantiomers elute (as one peak) switch effluent into the 250 μL sample loop of an injection valve. When the whole peak has been collected inject the contents of the sample loop onto column B with mobile phase B.

HPLC VARIABLES

Column: A 150 × 4.6 3 μm Supelcosil ODS; B 100 × 4.6 LKB EnantioPac
Mobile phase: A MeOH:water 60:40; B Isopropanol:20 mM pH 5.7 phosphate buffer 4:96
Column temperature: 28
Flow rate: A 0.7; B 0.4
Injection volume: 20
Detector: A UV 205; B UV 205

CHROMATOGRAM

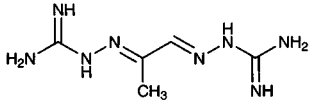
Retention time: 43 (11R,16S and 11S,16R from column A), 37 (11S,16R from column B), 55 (11R,16S from column B)

KEY WORDS

chiral; column-switching

REFERENCE
Roston,D.A.; Wijayaratne,R. Two-dimensional liquid chromatographic method for resolution of prostaglandin enantiomers, *Anal.Chem.*, **1988**, *60*, 948–950.

Mitoguazone



Molecular formula: $C_5H_{12}N_8$
Molecular weight: 184.20
CAS Registry No.: 459-86-9
Merck Index: 6299

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 10 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Hypersil BDS C8
Mobile phase: MeCN:buffer 11:89 (Buffer was 50 mM KH_2PO_4 containing 1 g/L sodium heptanesulfonate, adjusted to pH 3.0 with concentrated phosphoric acid.)
Column temperature: 40
Flow rate: 2
Injection volume: 20
Detector: UV 283

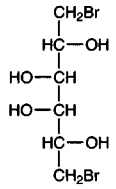
CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
comparison with capillary electrophoresis and TLC

REFERENCE
Thomson,C.E.; Gray,M.R.; Baxter,M.P. The use of capillary electrophoresis as part of a specificity testing strategy for mitoguazone dihydrochloride HPLC methods, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1103–1101.

Mitolactol



Molecular formula: $C_6H_{12}Br_2O_4$
Molecular weight: 307.97
CAS Registry No.: 10318-26-0
Merck Index: 6300

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 1 mL ice-cold MeOH, let stand at -20° for 20 min, centrifuge at 1500 g for 5 min. Remove a 1 mL aliquot of the supernatant and add it to 1 mL 5% diethyldithiocarbamate and 2 mL 50 mM pH 7.4 potassium phosphate buffer, heat at 50° for 1 h, extract with 5 mL chloroform. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute, inject an aliquot.

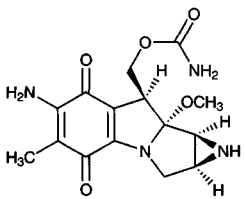
HPLC VARIABLES
Column: 250 × 4.6 5 μm Alltech CN
Mobile phase: Heptane:isopropanol:acetic acid 74.4:21.6:4
Flow rate: 1.3
Detector: UV 254

CHROMATOGRAM
Retention time: 9.9
Limit of detection: 500 nM

KEY WORDS
derivatization; plasma; human; mouse; pharmacokinetics

REFERENCE
Henner,W.D.; Furlong,E.A.; Kelley,S.L.; Rosowsky,A. Assay for mitolactol and its bifunctional alkylating metabolites in plasma, *J.Pharm.Sci.*, **1985**, *74*, 983–986.

Mitomycin



Molecular formula: $C_{15}H_{18}N_4O_5$
Molecular weight: 334.33
CAS Registry No.: 50-07-7
Merck Index: 6301

SAMPLE
Matrix: aqueous humor
Sample preparation: 100 μL Aqueous humor + 25 μL 5 μg/mL 4-aminoacetophenone in water, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: Microsorb C18
Column: 50 × 4.6 3 μm Microsorb C18 (Short-One)
Mobile phase: MeOH:water 28:72 containing $(NH_4)H_4PO_4$ adjusted to pH 7.0 with dilute ammonium hydroxide
Flow rate: 1.5
Injection volume: 100
Detector: UV 365

CHROMATOGRAM
Retention time: 2.3
Internal standard: 4-aminoacetophenone (2.7)
Limit of detection: 6.25 ng/mL

REFERENCE
Li,W.Y.; Seah,S.K.L.; Koda,R.T. Determination of mitomycin C in human aqueous humor and serum by high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *619*, 148–153.

SAMPLE
Matrix: aqueous humor, tissue
Sample preparation: Tissue. Add 5 mL cold MeCN to 200 mg conjunctiva or sclera, homogenize, sonicate for 30 min, centrifuge at 4° at 3000 rpm for 10 min, evaporate the organic layer to dryness under reduced pressure at 40°, reconstitute with 400 μL mobile phase, sonicate, filter, inject an aliquot. Aqueous humor. Add 4 mL ethyl acetate to 200 μL aqueous humor, stir. Sonicate for 30 min, centrifuge at 4° at 3000 rpm for 10 min, evaporate the organic layer to dryness under reduced pressure at 40°, reconstitute with 400 μL mobile phase, sonicate, filter, inject an aliquot.